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Bioremediation of Crude Oil and oily Sludge Contaminated soil and water

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For more information about this report, please contact

Microbial Biotechnology

T E R I

Darbari Seth Block

Habitat Place, Lodhi Road

New Delhi – 110 003 / India

Telephone +91 11 468 2111 or 468 2100

E-mail mailbox@teri.res.in

Fax +91 11 468 2144 or 468 2145

Web site www.teriin.org

Project Team

Team Members

Dr. Banwari Lal (Principal Investigator)

Mr. Vinod Kumar

Mr. Abu Swaleha

Mr. Samarjeet

Contents

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(Banwari Lal, Vinod Kumar, Abu Swaleya and Samarjeet)

1 Introduction

Oil spills are major menaces to the environment as they cause severe damage to surrounding ecosystems. During the Gulf War, several oil reservoirs were destroyed at sea, and the effect on marine life in their wake was devastating. Since crude oil is lighter than water, it floats on the sea surface and poses the threat of swift-spreading fire. Leave alone war time, oil spills are rather common in day to day activity of oil exploration companies at their drilling sites or oil unloading sites.

Also, crude oil is transported to various processing units through a network of terrestrial pipelines. These pipelines can get damaged, spewing out crude oil over a large acreage.

The damage could be attributed to poor maintenance, sabotage by insurgent elements (as recently witnessed in Assam, India), or even theft. Oil spills on land pose a three-fold menace of (1) fire hazards, (2) groundwater pollution due to percolation, and (3) air pollution due to evaporation.

Apart from accidental crude oil spills, oily sludge, a hydrocarbon waste generated in huge quantity in crude oil storage tanks also creates environment pollution. Oil companies need a well planned oily sludge management strategy. A straightforward approach may be dumping the oily sludge into sludge pits. Since, the possibility of seepage cannot be ruled out, the ideal sludge pit should incorporate a leachate collection system and a polymer lining to prevent percolation of contaminants into the groundwater. Such pits are not only way too expensive, but also needed in large numbers. Since there is a limit to the area available within tank farm area, alternative solutions for eradication of oily sludge have to be sought.

Biological organisms, usually bacteria and fungi, and occasionally plants have been utilized to reduce/eliminate toxic pollutants. These microorganisms either eat up the contaminants (mostly organic compounds), or assimilate them (heavy

metals), thus cleaning up the surrounding milieu. This biotechnological intervention – bioremediation – has been in use since the 1970s. It acquired global acceptance when the US Environmental Protection Agency and the Exxon Company demonstrated its effectiveness on Alaskan beaches contaminated by the *Valdez* oil spill.

After extensive research work, TERI has developed Oilzapper (crude oil and oily sludge degrading bacterial consortium) from various oil-eating bacterial cultures from the natural environment. These bacteria are multiplied under laboratory conditions, mixed with carrier materials, and packed into reusable polybags for convenient transportation. Utilizing Oilzapper to reclaim soil contaminated by crude oil spillage or oily sludge deposition is a very cost-effective and environmentally sound approach. The technique can be suitably modified to combat varying contaminants, as there is an immense diversity of bacteria to be tapped. The science of bioremediation thus acquires great significance in the area of research and development. Application of Oilzapper bacteria at IOCL's (Indian Oil Corporation Limited's) refineries provided encouraging results, on the basis of which larger sites were taken up and soon completely reclaimed.

The aim of the present study is to demonstrate the potential of Oilzapper (crude oil/oily sludge degrading bacterial consortium) to reclaim the crude oil contaminated land and manage oily sludge in tank farm area.

2 Bioremediation of oil contaminated land at Bowser unloading site near OCS3

Methods

Mass scale production of Oilzapper and its application on oil contaminated land at Bowser unloading site near OCS3

"*Oilzapper*" (a sludge degrading bacterial system) was grown in a fermenter on minimal salt medium. After 16 hours of growth cultures were harvested and mixed with a carrier material. The carrier based Oilzapper (containing 10^{10} cells/g carrier material) was filled in sterilized polythene bags and sealed (Figure 1). Sealed culture packets were kept in dark at 25°C for 7 days for curing. Cured culture packets were transported to Oil India Ltd., Duliajan and applied on oil contaminated land at Bowser unloading site near OCS3.

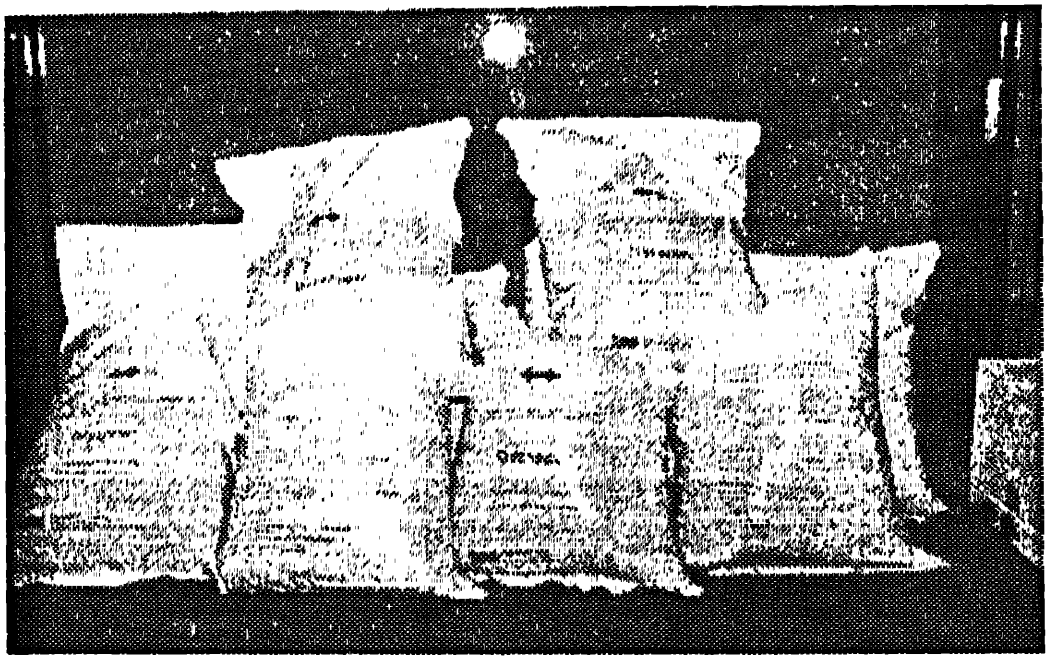


Figure 2.1. Oilzapper - A carrier based crude oil/oily sludge degrading bacterial consortium

Composition of crude oil biodegraded at Bowser unloading site near OCS3

The samples of Crude oil biodegraded at Bowser unloading site was collected and analysed before initiation of bioremediation work. The solvent extractable total petroleum hydrocarbon from crude oil was extracted using hexane, methylene chloride and chloroform. After evaporation of solvents, the amount of TPH recovered from 10 g of crude oil was quantified. The TPH recovered from crude oil was further fractionated into alkane, aromatic, asphaltene and NSO (nitrogen, sulphur, oxygen containing compounds) fractions with silicagel column. After TPH extraction, the residual crude oil (1 g) was heated at 600°C for five hours. After cooling, the amount of sediments/ash recovered from residual crude oil was quantified by gravimetrically.

Composition of TPH recovered from crude oil

The TPH recovered from crude oil was fractionated into alkane, aromatic asphaltene and NSO (nitrogen, sulfur and oxygen containing compound) with a silicagel column. The TPH was first dissolved in n-pentane and separated into soluble and insoluble fractions (asphaltanes). The soluble fraction was loaded on silica-gel column and eluted with different solvents. The alkane fraction was eluted with hexane, followed by the aromatic fraction that was eluted with benzene. The NSO fraction was eluted with chloroform and methanol. Alkane and aromatic fractions were concentrated by evaporation of solvents and then 0.2 µl of each was analyzed by gas-liquid chromatography (GLC Hewlett Packard, 5890 Series II) to identify all the compounds present in the alkane and aromatic fractions. In the alkane fraction, various alkane compounds were identified by matching the retention time with authentic standards. Simultaneously, the identification of each alkane compound was also confirmed by spiking the alkane fraction. A known amount of each standard alkane compound was added to the alkane fraction and injected into GLC (co-injection) and the peak area of each alkane compound increased as a result. Similarly, the polycyclic aromatic hydrocarbon compounds were identified by matching the retention time with an authentic standard and confirmed by spiking the sample with authentic standards.

Bioremediation of crude oil contaminated land at Bowser unloading site near OCS3

Crude oil contaminated land at Bowser unloading site near OCS 3 was selected for bioremediation. The land was contaminated with crude oil. Heavy dose of

Oilzapper and nutrients was applied on contaminated land. After application of Oilzapper, crude oil was mixed in soil by tilling of site. The tilling of site was also carried out at periodic intervals. The soil samples (10 samples) were collected from bioremediation site at zero time (just before initiation of bioremediation) and after two months. The samples were transported to the TERI laboratory at New Delhi for analysis. The solvent extractable total hydrocarbon was extracted from 10 g soil samples collected at zero time and after two months of initiation of the work. And thus the amount of TPH biodegraded at Bowser unloading site was estimated. The TPH recovered from samples (crude oil contaminated soil) were fractionated into alkane, aromatic, NSO and asphaltanes and the concentration of each fraction was estimated. The alkane and aromatic fractions were also analysed by GLC and degradation of individual alkane and aromatic compounds were quantified.

Results

Composition of crude oil biodegraded at Bowser unloading site near OCS3

Crude oil contaminated land at Bowser unloading site near OCS 3 was undertaken for bioremediation. Site was contaminated with heavy crude oil. The composition of heavy crude oil lying at bioremediation site shows 68% total petroleum hydrocarbon (TPH), 22% water and 10% sediments / inoganics (Table 2.1). TPH was further fractioned into various fractions by silicagel column showed 62% alkane fraction, 29% aromatic fraction, 4% NSO (Nitrogen, Sulphur, Oxygen containing compounds) fraction and 5% asphaltene fraction (Table 2.1).

Table 2.1 Composition of heavy oil biodegraded at Bowser unloading site

Constituents	Composition (%)
Total petroleum hydrocarbon	68
Water	22
Sediments / Inorganic	10
Alkane fraction in TPH	62
Aromatic fraction in TPH	29
NSO fraction in TPH	4
Asphaltene fraction in TPH	5

Bioremediation of crude oil contained land at Bowser unloading site near OCS 3

Crude oil contaminated land at Bowser unloading site was undertaken for bioremediation. Heavy dose of Oilzapper plus nutrient was applied on contaminated land at bioremediation site. At zero time (just before application of Oilzapper at site), the crude oil (TPH) contamination in soil in the month of November 1999 was 38.47%. With treatment of crude oil contaminated land with Oilzapper plus nutrient exhibited that the crude oil contamination in soil reduced from 38.47% to 13.82% in two months (January, 2000) which revealed 64% biodegradation of crude oil in two months (Figure 2.2). The biodegradation of various fraction of crude oil was also estimated. Out of 64% biodegradation of crude oil (TPH), biodegradation of alkane fraction was accounted for 32.2%, aromatic fraction 26.6% and NSO plus Asphaltene fraction 5.2% in two months (Table 2.2). The GLC fingerprinting of biodegradation of alkane and aromatic fractions of crude oil are shown in Figures 2.3 and 2.4

respectively. Bioremediation work at this site was discontinued after 2 months (after January, 2000) due to water logging conditions due to heavy rains as this site was situated in low line area.

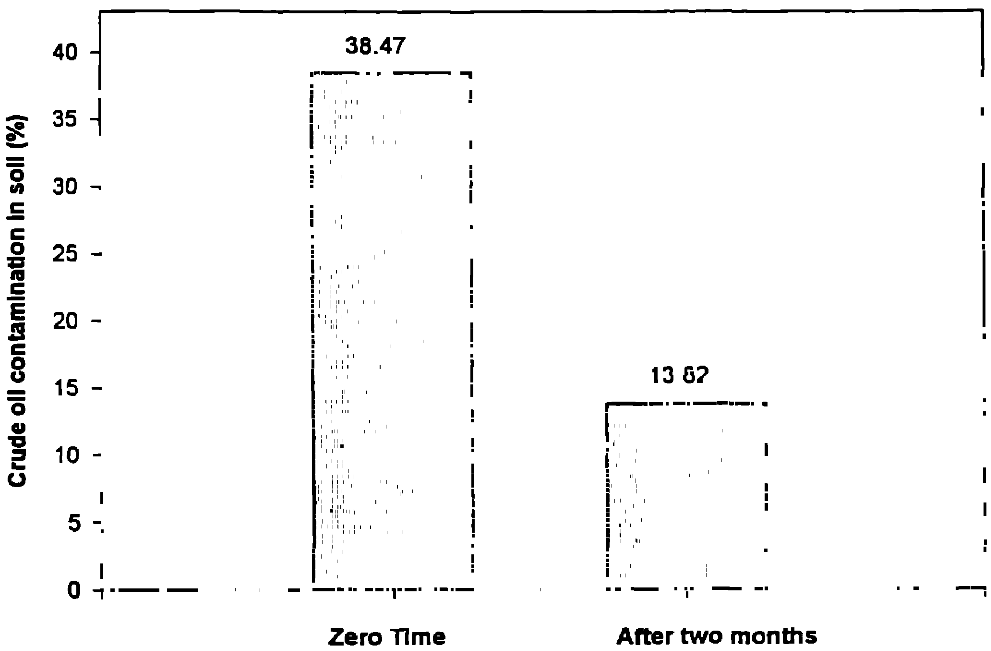


Figure 2.2 Bioremediation of crude oil contaminated land at Bowser unloading site near OCS 3

Table 2.2 On-site biodegradation of various fraction of crude oil in contaminated soil at Bowser unloading site

Fraction of Crude Oil	Biodegradation (%)
Total petroleum hydrocarbon	68
Alkane fraction of crude oil	32.2
Aromatic fraction of crude oil	26.6
NSO + Asphaltene fraction of crude oil	5.2

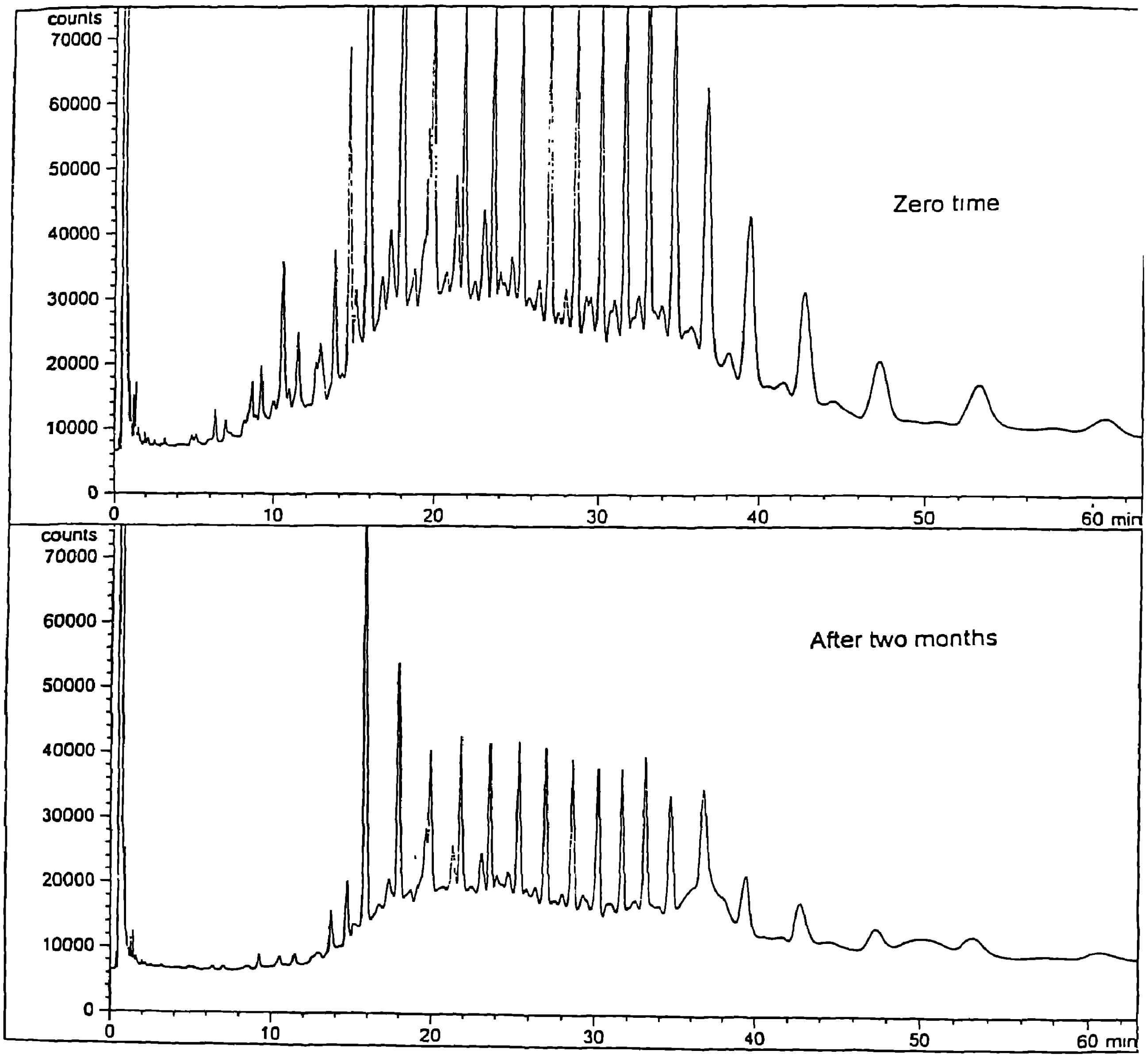


Figure 2.3 GLC fingerprinting of biodegradation of alkane fraction of crude oil in contaminated soil at Bowser unloading site near OCS 3

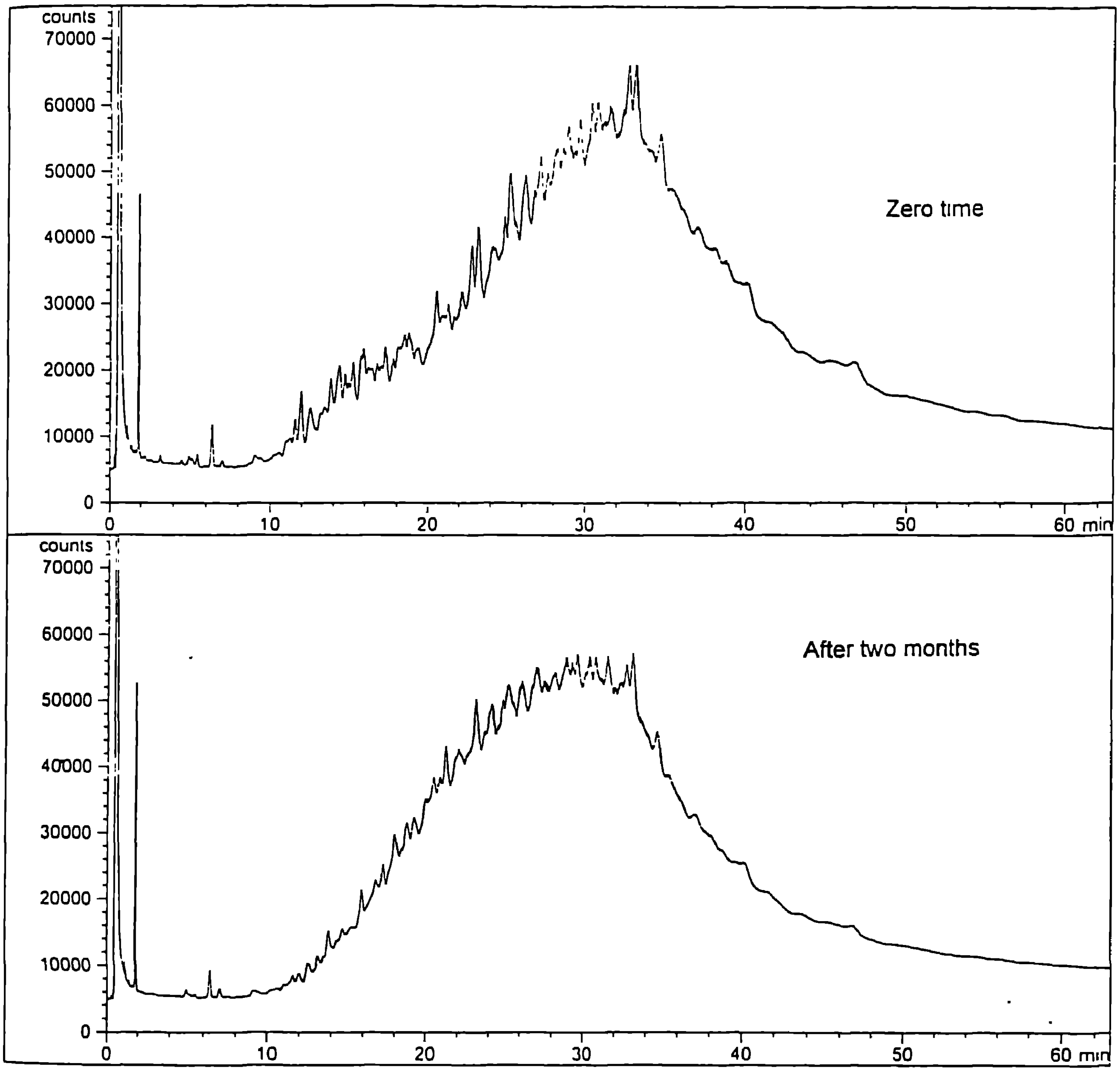


Figure 2.4 GLC fingerprinting of biodegradation of aromatic fraction of crude oil in contaminated soil at Bowser unloading site near OCS 3

3 Bioremediation of oily sludge in tank farm area

Methods

Selection of site for bioremediation of oily sludge

At Oil India Ltd., Duliajan, the site for bioremediation of oily sludge was selected in sludge pits situated in tank farm area of tank No.1, 2, 4, 5 and 6. Bioremediation of oily sludge in sludge pits 1, 5 and 6 were initiated in the month of November 1999, while in pit 2 and 4 work was initiated in the month of April 2000. Each pit was containing approximately 150-200 tonnes sludge. In each pit, approximately 150-200 kg Oilzapper was applied on oily sludge and nutrients were also sprayed on sludge along with Oilzapper.

Composition of oily sludge biodegraded in sludge pits

Oily sludge collected from sludge pits 1, 2, 4, 5 and 6 was analysed before initiation of bioremediation work. The solvent extractable total petroleum hydrocarbon from oily sludge was extracted using hexane, methylene chloride and chloroform. After evaporation of solvents, the amount of TPH recovered from 10 g of oily sludge was quantified. The TPH recovered from oily sludge was further fractionated into alkane, aromatic, asphaltene and NSO (nitrogen, sulphur, oxygen containing compounds) fractions with silicagel column. After TPH extraction, the residual oily sludge (1 g) was heated at 600°C for five hours. After cooling, the amount of sediments/ash recovered from residual oily sludge was quantified by gravimetrically.

Composition of TPH recovered from oily sludge

The TPH recovered from oily sludge was fractionated into alkane, aromatic asphaltene and NSO (nitrogen, sulphur and oxygen containing compound) with a silicagel column. The TPH was first dissolved in n-pentane and separated into soluble and insoluble fractions (asphaltene). The soluble fraction was loaded on silica-gel column and eluted with different solvents. The alkane fraction was eluted with hexane, followed by the aromatic fraction that was eluted with benzene. The NSO fraction was eluted with chloroform and methanol. Alkane and aromatic fractions were concentrated by evaporation of solvents and then

0.2 μl of each was analysed by gas-liquid chromatography (GLC Hewlett Packard, 5890 Series II) to identify all the compounds present in the alkane and aromatic fractions. In the alkane fraction, various alkane compounds were identified by matching the retention time with authentic standards. Simultaneously, the identification of each alkane compound was also confirmed by spiking the alkane fraction. A known amount of each standard alkane compound was added to the alkane fraction and injected into GLC (co-injection) and the peak area of each alkane compound increased as a result. Similarly, the polycyclic aromatic hydrocarbon compounds were identified by matching the retention time with an authentic standard and confirmed by spiking the sample with authentic standards.

Bioremediation of oily sludge

Sludge pits 1, 2, 4, 5 and 6 were undertaken for bioremediation of oily sludge by Oilzapper. Oilzapper was applied on oily sludge in each pit. After applying Oilzapper soil was also applied in pits 1,2 and 6 for providing air pockets in sludge for the Oilzapper bacteria. After applying soil in sludge pits tilling was done for mixing of Oilzapper and oily sludge. However, pit 4 and 5 containing water and oily sludge and thus soil was not applied. The zero time sampling (10 samples) from each pit was done just after application of Oilzapper. The tilling in all six pits were carried out at periodic intervals. The sampling (10 samples from each pit) from all five pits were also carried out after two months interval from initiation of bioremediation work. The samples were transported to the laboratory at TERI, New Delhi for analysis. Oily sludge was heterogeneously distributed in the samples collected from sludge pits at zero time and after two months intervals and therefore, the samples were homogenised, and then solvent extractable total petroleum hydrocarbon (TPH) from 10 g sample was extracted with hexane methylene chloride and chloroform (80 ml each) consecutively. After extraction, all three extracts obtained by hexane, methylene chloride and chloroform were pooled and dried at room temperature by evaporation of solvent under gentle nitrogen stream in a fumehood. After solvent evaporation, the amount of TPH recovered from 10 g sample collected from pits at zero time and after two months intervals were determined by simple gravimetry. The difference in TPH concentration of 10 g sample of zero time and after different time intervals, revealed the amount of sludge (TPH) biodegraded in sludge pits.

Biodegradation of various fractions of oily sludge in sludge pits

The TPH recovered from 10 samples collected at zero time and after intervals of two months was further fractionated into alkane, aromatic, NSO and asphaltene fractions by silicagel column as above. The concentration of these fractions was estimated by gravimentry. The alkane and aromatic fractions of oily sludge recovered from samples at zero time and after intervals of two months were also analysed by GLC and degradation of these fractions were estimated by data obtained by GLC analysis.

Results

Composition of oily sludge Biodegraded in sludge pits

Before initiation of bioremediation in sludge pit 1, the composition of oily sludge was estimated. The composition of oily sludge shows 57% solvent extractable total petroleum hydrocarbon (TPH), 15.8% water content, 20.2% sediment/ash and 7% organic matter (Table 3.1).

Table 3.1 Composition of oily sludge of sludge pit 1

Constituents	Results
Total petroleum hydrocarbon (solvent extractable)	57.0
Water content	15.8
Organic matter	7.0
Sediments/ash	20.2

The TPH was further fractionated into various fractions showed 49% alkane fraction, 28% aromatic fraction, 9% NSO fraction and 14% asphaltene fraction (Table 3.2).

Table 3.2 Composition of TPH recovered from oily sludge lying in pit 1

Fraction of TPH	Percent (%)
Alkane fraction	49%
Aromatic fraction	28%
NSO fraction	9%
Asphaltene fraction	14%

The composition of oily sludge of sludge pit 5 was estimated before initiation of bioremediation work. Oily sludge of pit 5 contained 58% solvent extractable TPH, 22% water content, 9% organic matter (wax and other organic waste) and 11% sediment, ash and inorganic waste (Table 3.3). The TPH extracted from oily

sludge was further fractionated into different fractions. The composition of TPH revealed 47% alkane fraction, 34% aromatic fraction, 8% NSO fraction and 11% asphaltene fraction (Table 3.4).

Table 3.3 Composition of oily sludge of sludge pit 5

Constituents	Result
Solvent extractable total petroleum hydrocarbon	58%
Water content	22%
Organic matter	9%
Sediment/ash content	11%

Table 3.4 Composition of TPH recovered from oily sludge lying in pit 5

Fractions of TPH	Result
Alkane fraction	47%
Aromatic fraction	34%
NSO fraction	8%
Asphaltane	11%

The composition of oily sludge of pit 6 was also estimated just before bioremediation of oily sludge. Oily sludge of pit 6 contained 45% solvent extractable TPH, 17% water content, 12% organic matter and 26% sediments/ash content (Table 3.5). The TPH extracted from oily sludge was further fractionated into different fractions. The composition of TPH revealed 43% alkane fraction, 30% aromatic fraction, 9% NSO fraction and 18% asphaltane fraction (Table 3.6).

Table 3.5 Composition of oily sludge of sludge pit 6

Constituents	Result
Solvent extractable total petroleum hydrocarbon	45%
Water content	17%
Organic matter	12%
Sediment/ash content	26%

Table 3.6 Composition of TPH recovered from oily sludge lying in sludge pit 6

Constituents	Result
Alkane fraction	43%
Aromatic fraction	30%
NSO fraction	9%
Asphaltane	18%

Composition of oily sludge lying in sludge pit 2 (crude oil tank 2) was estimated at zero time (just before application of Oilzapper). The composition of oily sludge shows 48% solvent extractable total petroleum hydrocarbon (TPH), 20% water, 14% organic matter and 18% sediment/ash content (Table 3.7).

Table 3.7 Composition of oily sludge of sludge pit 2

Constituents	Results (%)
Total petroleum hydrocarbon (solvent extractable	48
Water content	20
Organic matter	14
Sediment/ash	18

The constituents of TPH of oily sludge from sludge pit 2 showed 47.6% alkane fraction, 43.4% aromatic fraction, 4% NSO fraction and 5% asphaltene fraction (Table 3.8).

Table 3.8 Constituents of TPH recovered from oily sludge lying in pit 2

Fractions of TPH	Percent (%)
Alkane fraction	47.6
Aromatic fraction	43.4
NSO fraction	4.0
Asphaltene fraction	5.0

Composition of oily sludge of sludge pit 4 was also estimated at zero time. Oily sludge of sludge pit 4 contained 56% solvent extractable TPH, 16% water, 13% organic matter and 15% sediments/ash content (Table 3.9). Constituents of TPH of oily sludge of sludge pit 4 showed 59.2% alkane fraction, 38.6% aromatic fraction, 0.8% NSO fraction and 1.4% asphaltene fraction (Table 3.10)

Table 3.9 Composition of oily sludge of sludge pit 4

Constituents	Results (%)
Total petroleum hydrocarbon (solvent extractable	56
Water content	16
Organic matter	13
Sediment/ash	15

Table 3.10 Constituents of TPH recovered from oily sludge lying in pit 4

Fractions of TPH	Percent (%)
Alkane fraction	59.2
Aromatic fraction	38.6
NSO fraction	0.8
Asphaltene fraction	1.4

Bioremediation of oily sludge in sludge pits 1, 2, 4, 5 and 6

Bioremediation of oily sludge in sludge pits 1, 5 and 6 situated in tank dykes of tank 1, 5 and 6 was initiated in the month of November 1999. However, bioremediation of oily sludge in pits 4 and 5 were initiated in the month of April 2000. Heavy dose of Oilzapper and nutrients was applied on oily sludge in all five sludge pits. In sludge pit 1 at zero time (just after application of Oilzapper), the oil content in sludge (solvent extractable TPH) was 41.06%. With application of Oilzapper and nutrients, the oil content in sludge reduced from 41.06% to 8.91% after four months and 2.25% after one year (figure 3.1) which resulted in 78.3% biodegradation of oily sludge in four months and 96.9% biodegradation in a year. In sludge pit 5, the oil content in sludge was recorded 37.10% at zero time. Treatment of oily sludge with Oilzapper and nutrients showed that the oil content in sludge reduced from 37.10% to 18.20% after four months and 4.85% after one year (figure 3.1) which revealed 50.9% biodegradation of oily sludge in four months and 86.9% biodegradation in one year. Similarly, with application of Oilzapper and nutrients, the oil content in oily sludge of sludge pit 6 reduced from 36.4% to 12.08% in four months and 1.75% in one year (figure 3.1) which resulted in 66.8% biodegradation of oily sludge in four and 95.1% biodegradation in one year (Figure 3.1). Figure 3.2 shows after biodegradation of total petroleum hydrocarbon in sludge pit 1.

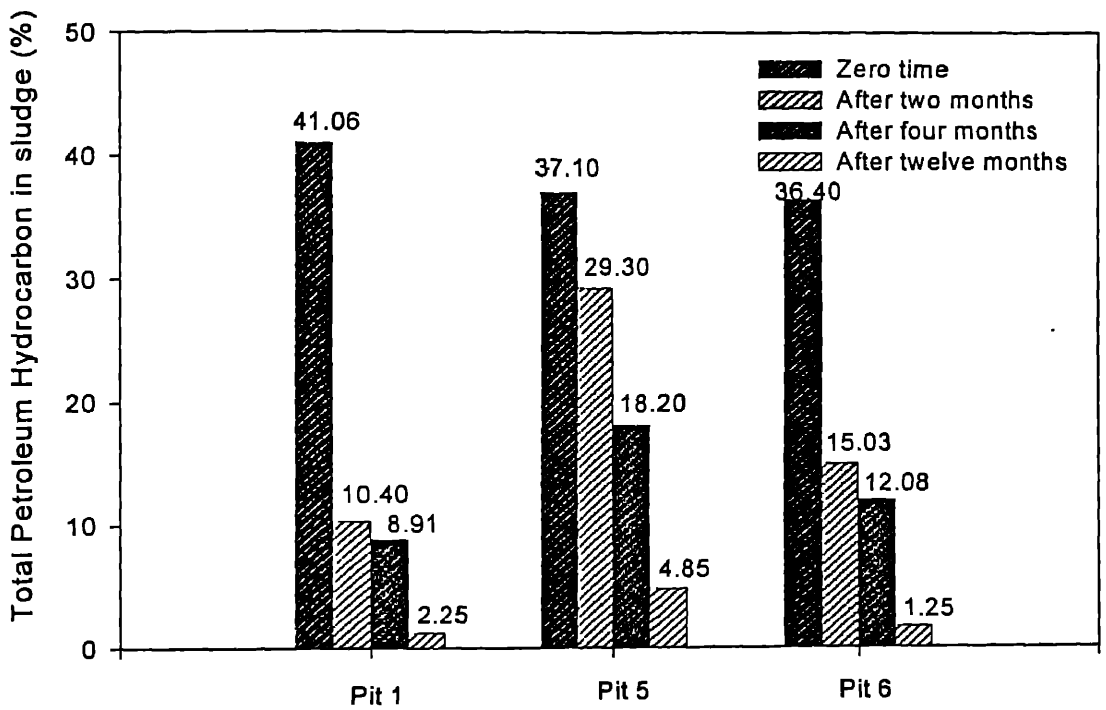


Figure 3.1 Biodegradation of oily sludge in sludge pit s

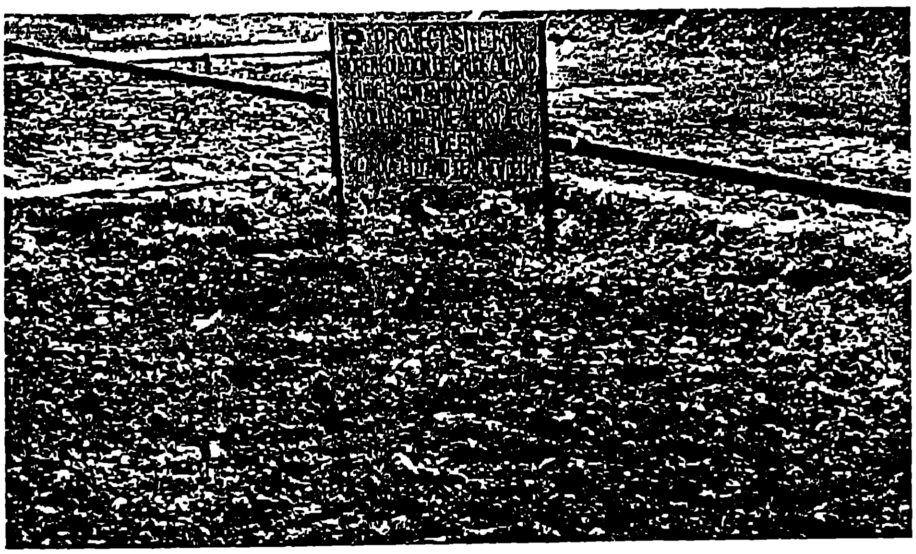


Figure 3.2 Bioremediation of oily sludge in sludge pit 1 situated in tank

Bioremediation of oily sludge in sludge pit 2 and 4 were initiated in the month of April, 2000. In the month of April 2000, the oil content (TPH) in oily sludge of pit 2 was 29.78. Treatment of oily sludge with Oilzapper revealed that oil content in oily sludge reduced from 29.78% to 6.66% in six months (October 2000), which resulted 77.6% biodegradation (figure 3.3). Similarly in the month of April 2000 the oil content in sludge of pit 4 was 32.16%. Treatment of this oily sludge by Oilzapper resulted that oil content in sludge reduced from 32.16% to 6.56% in six months (figure 3.3) which showed 79.6% biodegradation of oil content.

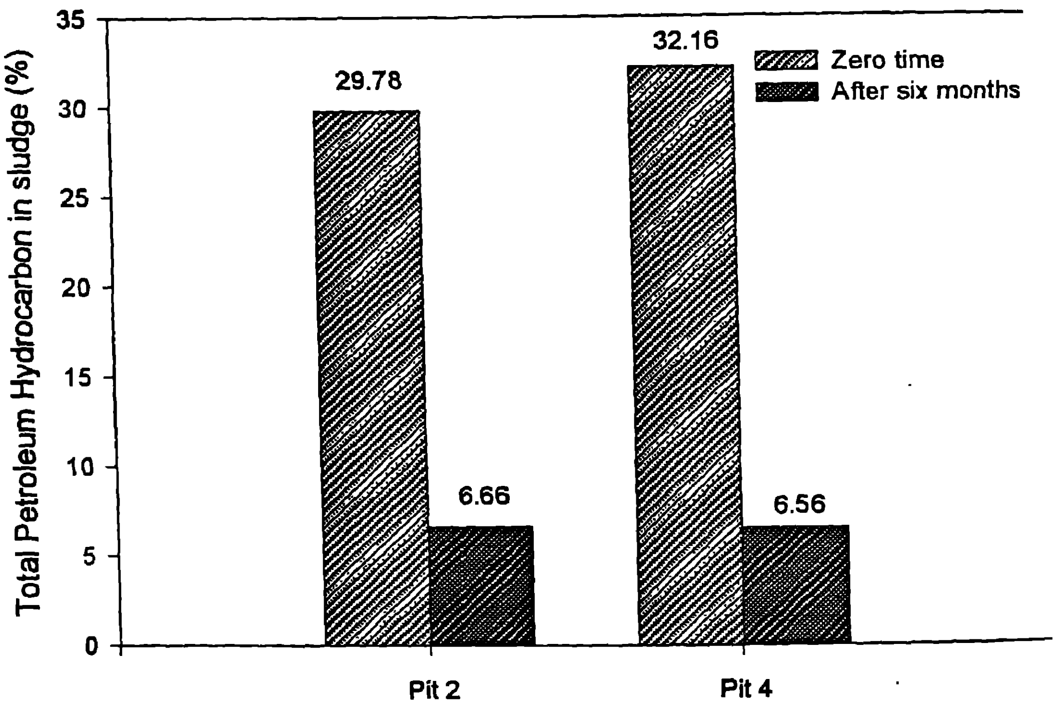


Figure 3.3 Biodegradation of oily sludge in sludge pits

Biodegradation of various fractions of oily sludge

In sludge pit 1, out of 96.9% biodegradation of oil content in a year, the biodegradation of alkane fraction was accounted for 48.10%, aromatic fraction 27.8%, NSO fraction 8.1% and asphaltene fraction 12.9% (Table 3.11). GLC fingerprinting of biodegradation of alkane and aromatic fractions of oily sludge of pit 1 is shown in figure 3 .4 and 3.5 respectively.

Table 3.11 Biodegradation of various fractions of oily sludge in one year

Fractions of oily sludge	Biodegradation (%)		
	Pit 1	Pit 5	Pit 6
Total petroleum hydrocarbon	96.9	86.9	95.1
Alkane fraction	48.1	45.7	42.2
Aromatic fraction	27.8	32.3	29.0
NSO fraction	8.1	6.9	7.9
Asphaltene fraction	12.9	2.0	16.0

In sludge pit 5, out of 86.9% biodegradation of oil content in one year, the alkane fraction was 45.7%, aromatic fraction 32.3%, NSO fraction 6.9% and asphaltene fraction 2.0% (Table 3.11). GLC fingerprinting of biodegradation of alkane and aromatic fraction of oily sludge of sludge pit 5 is shown in Figure 3.6 and 3.7 respectively. In sludge pit 6, total 95.6% of oily sludge was biodegraded in one year. The biodegradation of alkane, aromatic, NSO and asphaltene fractions were accounted for 42.2%, 29.0%, 7.9% and 16.0% respectively in one year (table 3.11). GLC analysis of biodegradation of alkane and aromatic fraction of oily sludge in sludge pit 6 is shown in figure 3.8 and 3.9 respectively. Similarly in sludge pit 6, biodegradation of total petroleum hydrocarbon was 77.6% in six months. Biodegradation of alkane, aromatic, NSO and asphaltene fractions of oily sludge was 44.2%, 29.8%, 1.2% and 2.4% respectively in six months (Table 3.12). However, in sludge pit 4, 79.6% TPH of oily sludge was biodegraded in six months. In this sludge pit biodegradation of alkane fraction of oily sludge was 57.9%, aromatic fraction was 21.0% in six months. However, biodegradation of NSO and asphaltene fractions of oily was negligible in sludge pit 4 (Table 3.12).

Table 3.12 Biodegradation of various fractions of oily sludge in six months

Fractions of oily sludge	Biodegradation (%)	
	Pit 2	Pit 4
Total petroleum hydrocarbon	77.6	79.6
Alkane fraction	44.2	57.9
Aromatic fraction	29.8	21.0
NSO fraction	1.2	-
Asphaltene fraction	2.4	0.6

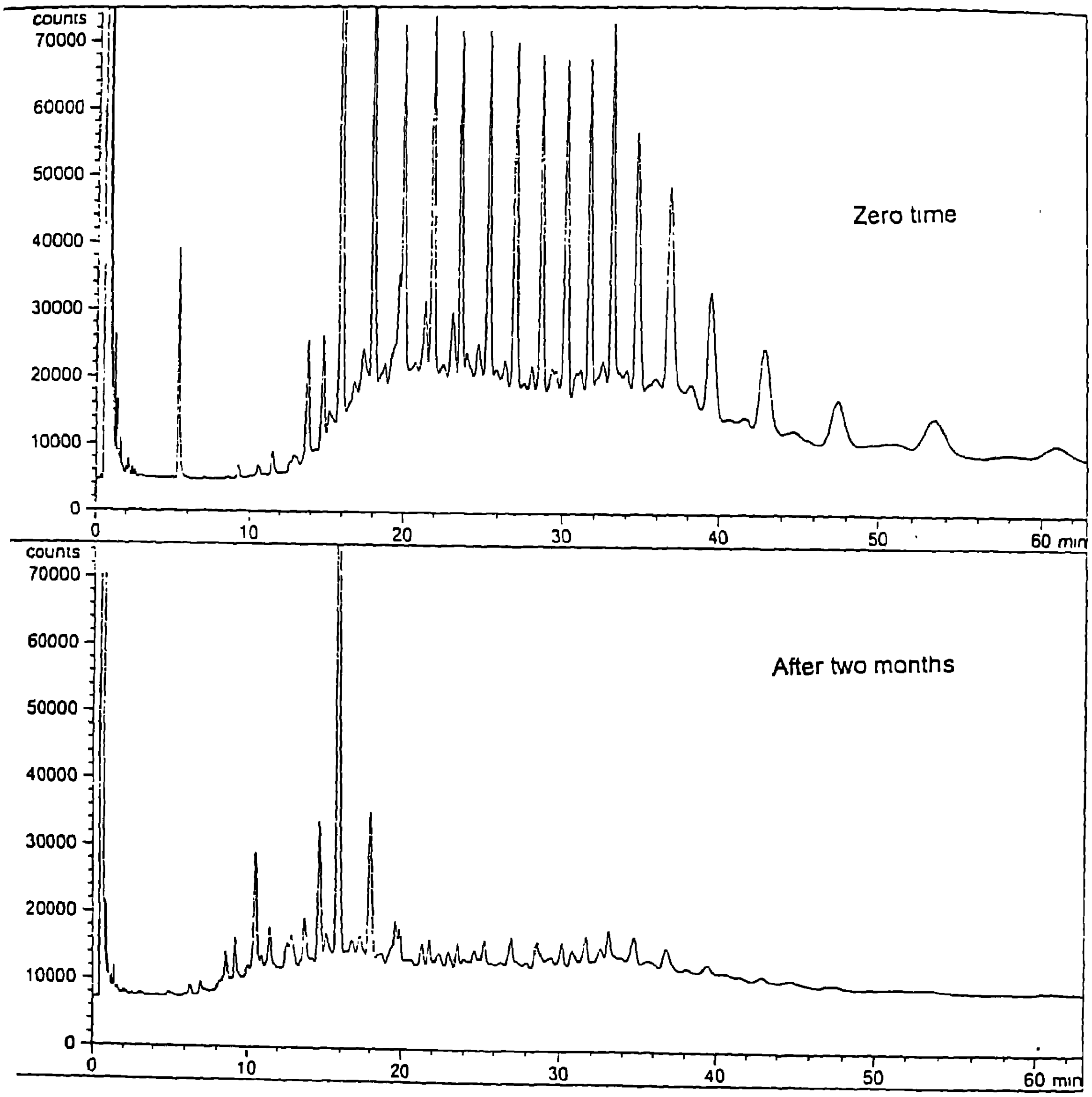


Figure 3.4 GLC fingerprinting of on site bioremediation of alkane fraction of oily sludge in sludge pit 1

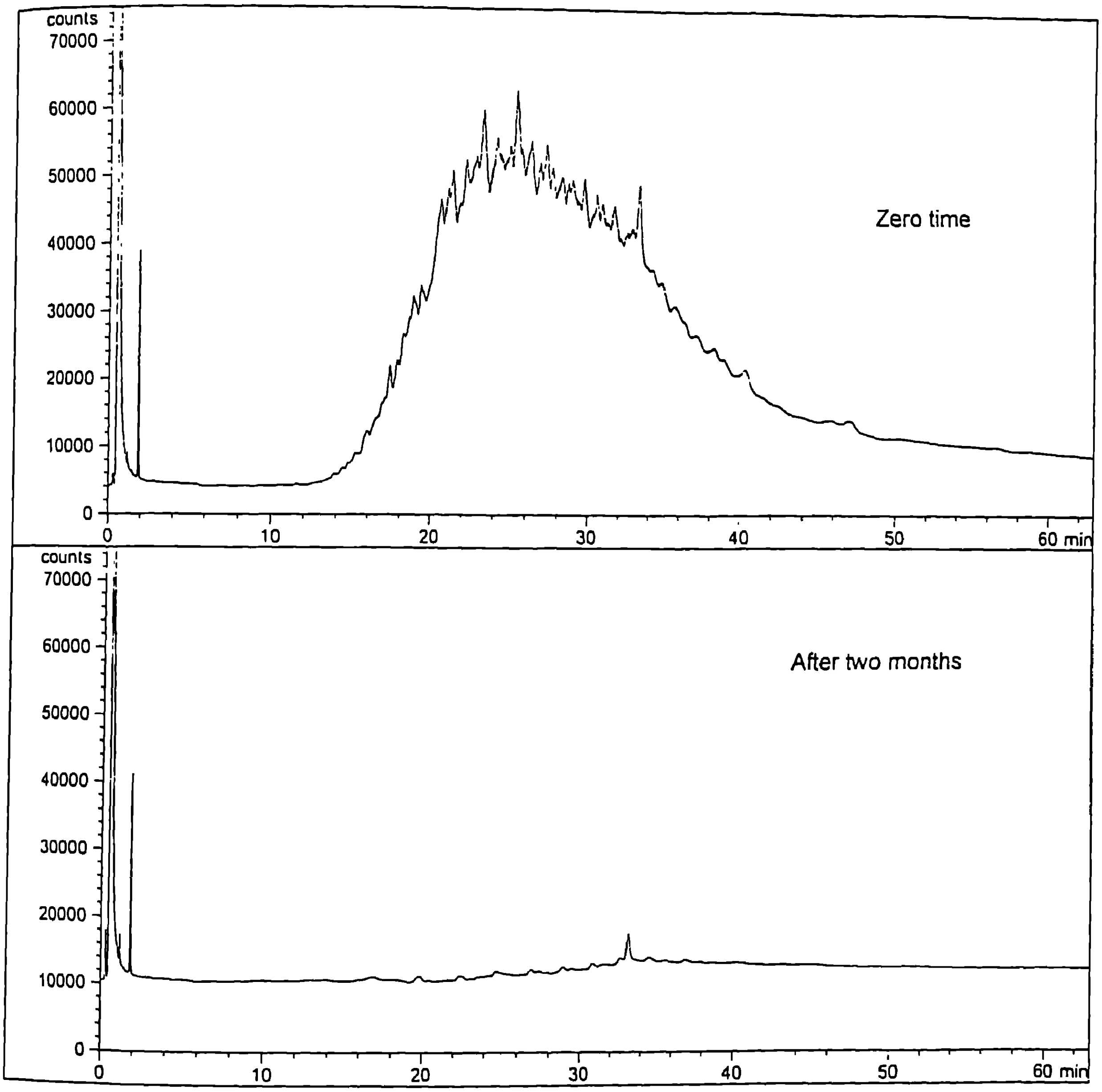


Figure 3.5 GLC fingerprinting of on site bioremediation of aromatic fraction of oily sludge in sludge pit 1

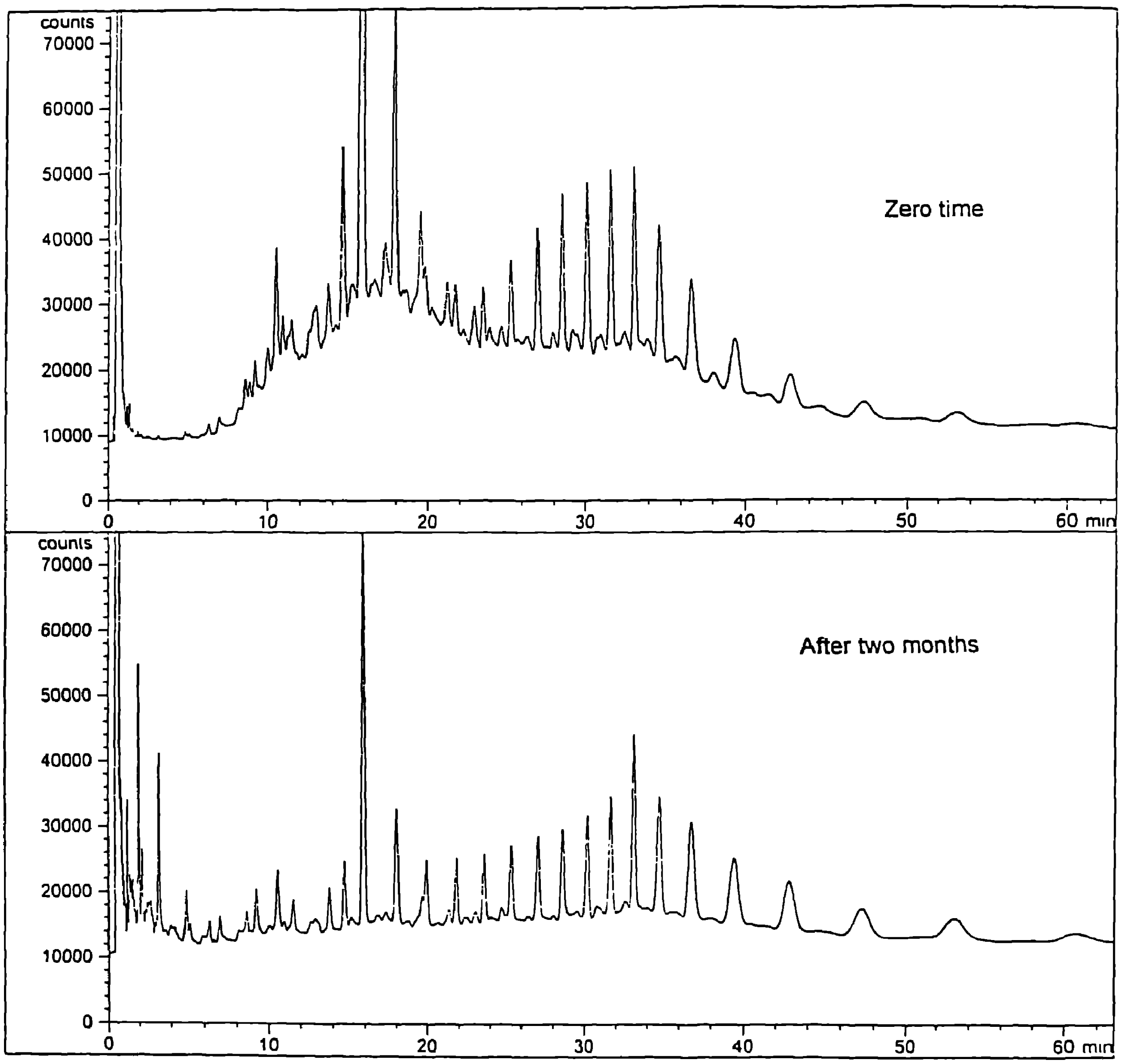


Figure 3.6 GLC fingerprinting of onsite bioremediation of alkane fraction of oily sludge in sludge pit 5

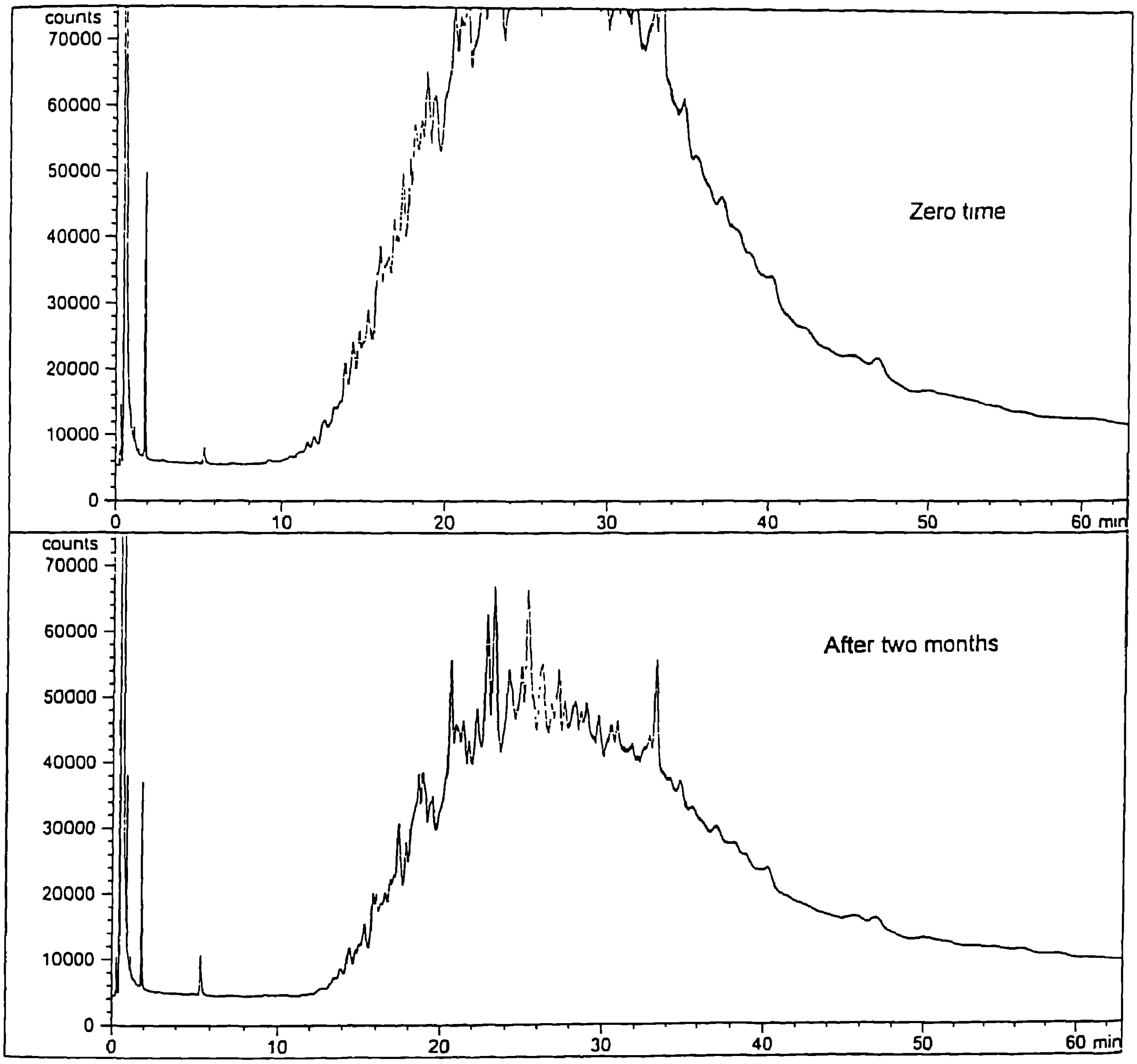


Figure 3.7 GLC fingerprinting of onsite bioremediation of aromatic fraction of oily sludge in sludge pit 5

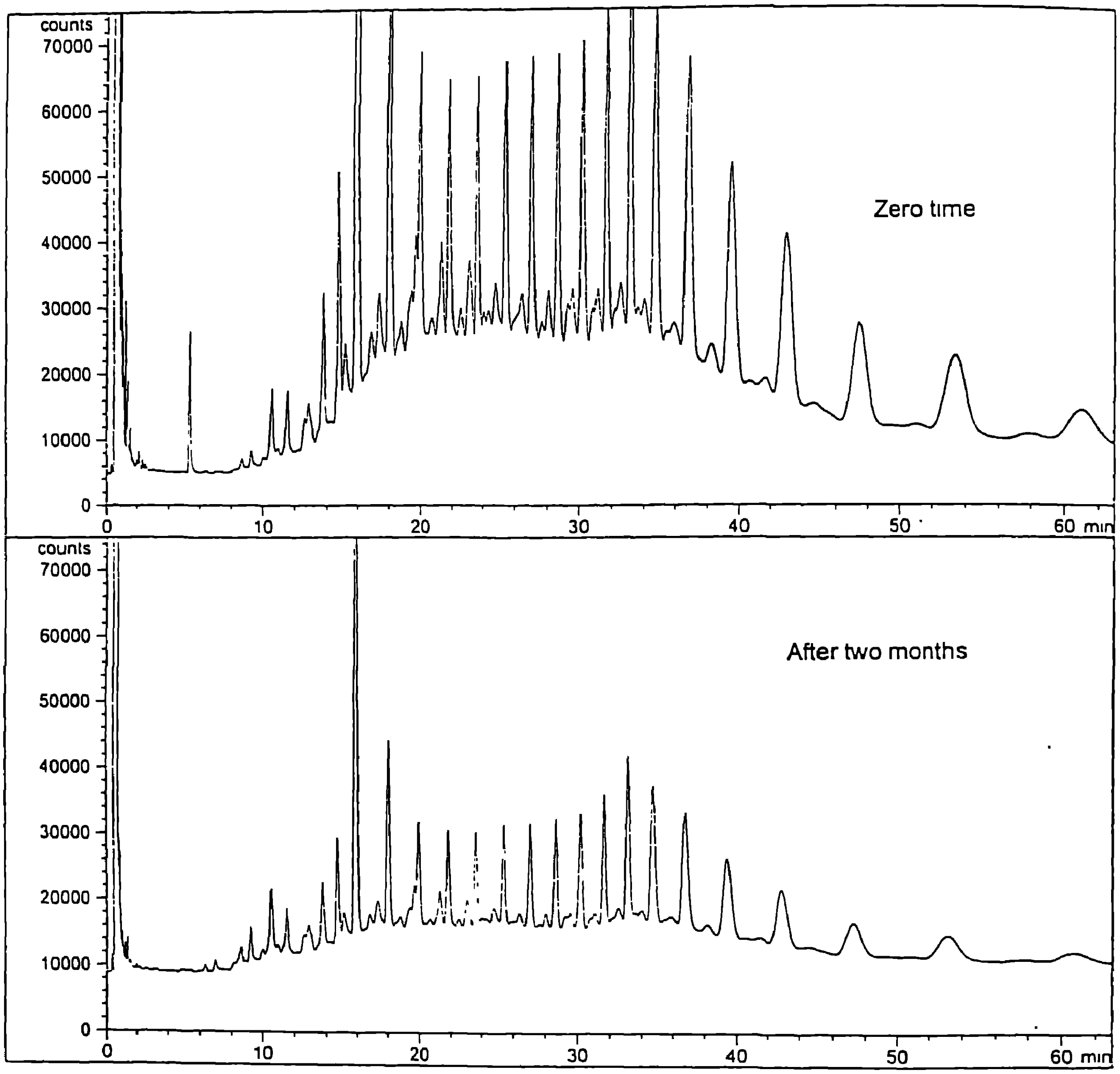


Figure 3.8 GLC fingerprinting of onsite bioremediation of alkane fraction of oily sludge in sludge pit 6

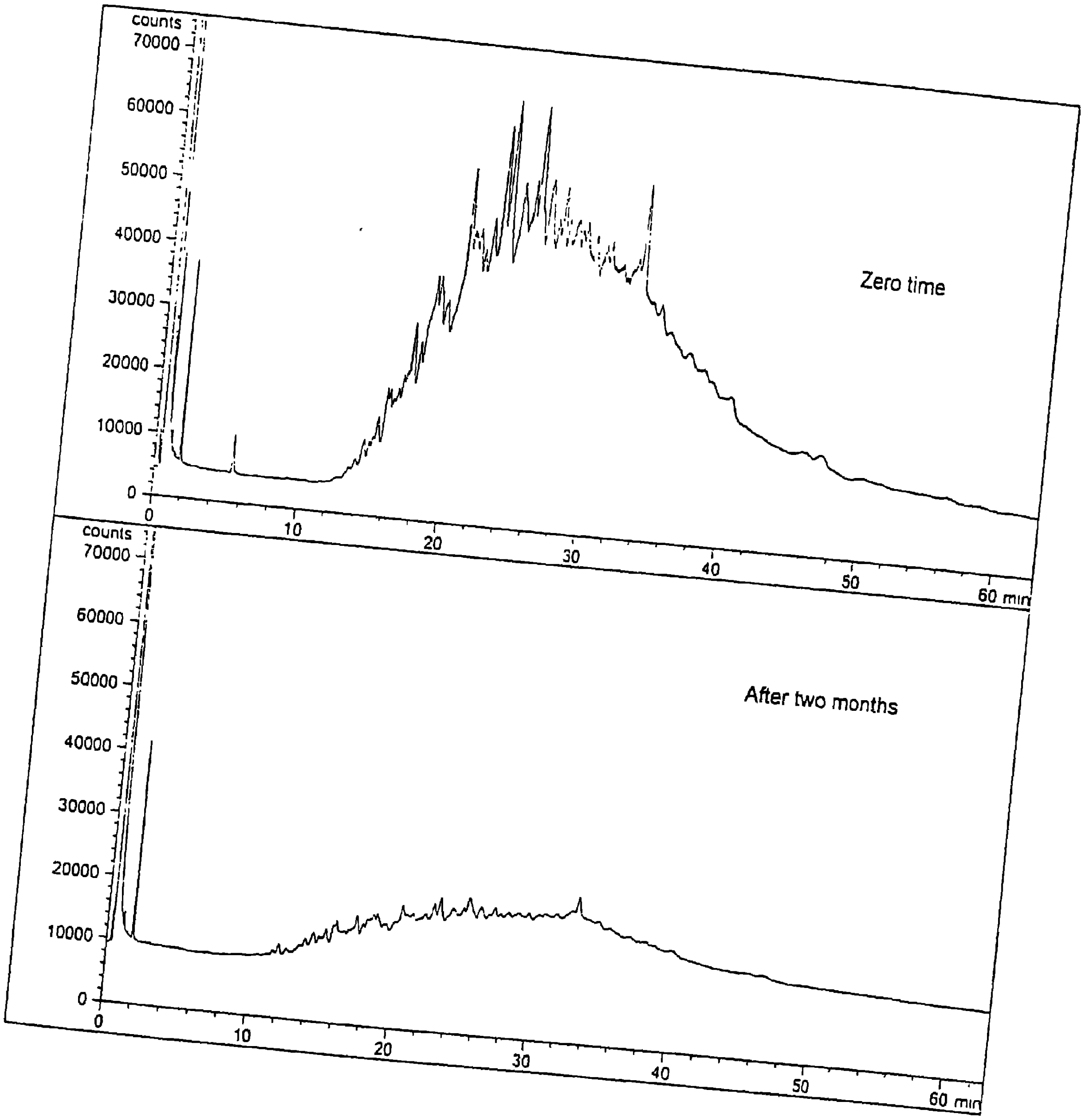


Figure 3.9 GLC fingerprinting of onsite bioremediation of aromatic fraction of oily sludge in sludge pit 6

4 Bioremediation of water contaminated with crude oil

Methods

Selection of site for bioremediation of water contaminated with crude oil

Water (formation water) pit near Joypur OCS was selected for bioremediation. Pit size was approximately 100m x 30m. Water in pit was contaminated with crude oil. A thick layer of crude oil (approximately 4 inch thick) was floating on surface water.

Composition of crude oil biodegraded in water pit near Joypur OCS

At zero time (just before application of Oilzapper in water pit) sample of crude oil floating on surface water was collected. Solvent extractable total petroleum hydrocarbon from 10g sample was extracted using hexane, methylene chloride and chloroform. After evaporation of solvents, the amount of TPH recovered from 10g sample was quantified. TPH recovered from 10g sample was further fractionated into alkane, aromatic, NSO and asphaltene fractions as described above. After TPH extraction from sample, the residual of sample was heated at 600^{0} C for five hours. After cooling, the amount of sediments/ash obtained from residual sample was quantified.

Bioremediation of water contaminated with crude oil

In the month of February 2000, a heavy dose of Oilzapper (150 kg) and nutrients (100 litres) was applied in one-third portion of pit. Oilzapper and nutrients were mixed with crude oil floating on surface water by using big bamboo sticks. In the month of October, 2000 Oilzapper was applied again on contaminated water. A boat was used to apply Oilzapper and nutrients in the water pit. At zero time (in February, 2000) just before application of Oilzapper and nutrients, ten samples of crude oil were collected from the pit. At the opposite end of this pit, a control was maintained. Crude oil samples (10 samples) were also collected from control. Samples were also collected in the

months of April 2000, October 2000, December 2000 and February 2001. The composition of crude oil collected from contaminated water was estimated.

Biodegradation of various fractions of crude oil

Crude oil samples collected from water pit at Joypur OCS at zero time and after two months of application of Oilzapper was fractionated into alkane, aromatic, NSO (Nitrogen, sulphur, oxygen containing compound) and asphaltene as described above. The relative concentration of these fractions in crude oil collected was estimated. *In situ* biodegradation of alkane and aromatic fractions was also estimated by gas liquid chromatography as described in chapter 2.

Results

Composition of crude oil biodegraded in water pit near Joypur OCS

Crude oil samples collected from water pit near Joypur OCS contained 50.88% TPH (solvent extractable oil), 27.12% water, 19% organic carbon and 3% sediments / ash content. Composition of TPH was also estimated by fraction of silicagel column. TPH recovered from samples contained 58% alkane fraction, 32% aromatic fraction, 4% NSO fraction and 6% asphaltene fraction (Table 4.1).

Table 4.1 Composition of crude oil biodegraded in water pit near Joypur OCS

Constituent of crude oil	Fractions (%)
Solvent extractable TPH	50.88
Water	27.12
Organic Carbon	19
Sediments / ash	3
Alkane fraction of TPH	58
Aromatic fraction of TPH	32
NSO fraction	4
Asphaltene fraction	6

Bioremediation of crude oil contaminated water near Joypur OCS

Water (formation) pit contaminated with crude oil was selected for bioremediation. In one third of this pit, Oilzapper and nutrients were applied on crude oil layer floating on water. At zero time (before application of Oilzapper in water) TPH in oil floating on water was 50.88%. With application of Oilzapper in this water pit revealed that TPH in oil floating on surface water reduced from 50.88% to 0.46% in fourteen months (figure 4.1) which showed 99%

biodegradation of TPH. Out of 99% biodegradation of floating oil, degradation of alkane fraction was accounted for 57.1% aromatic fraction was 55%, NSO fraction was 3% and asphaltene fraction was 3.9%. With application of Oilzapper, the crude oil was completely biodegraded in one third portion of water pit. The GLC fingerprinting of biodegradation of alkane and aromatic fraction of crude oil is shown in Figure 4.3 and 4.4 respectively. Figure 4.2 shows cleaned up of crude oil contaminated water in a pit near Joypur OCS.

Table 4.2 Biodegradation of various fractions of oil floating on surface water

Fractions of oil	Degradation (%)
TPH	99
Alkane fraction of TPH	57.1
Aromatic fraction of TPH	35
NSO fraction of TPH	3
Asphaltene fraction of TPH	3.9

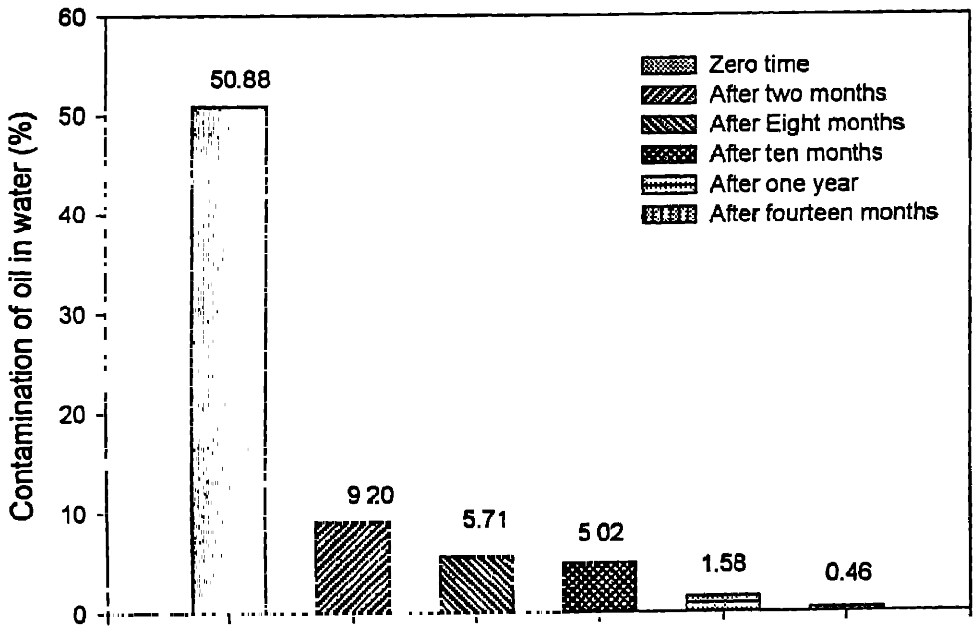
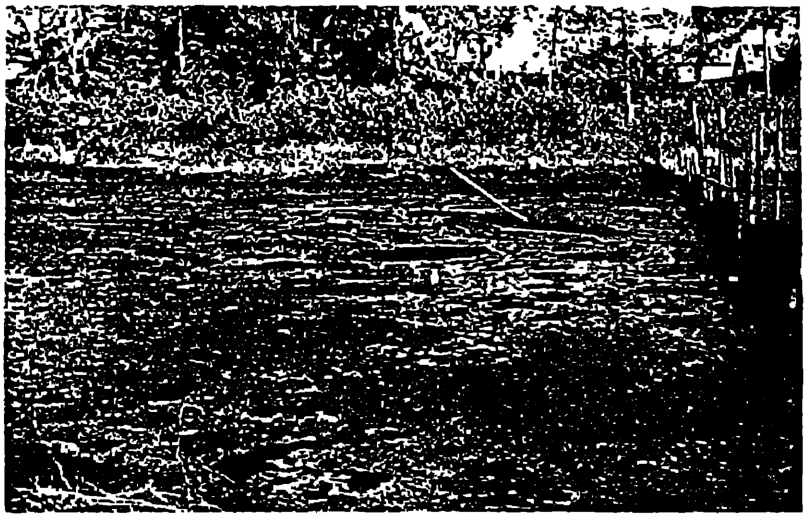
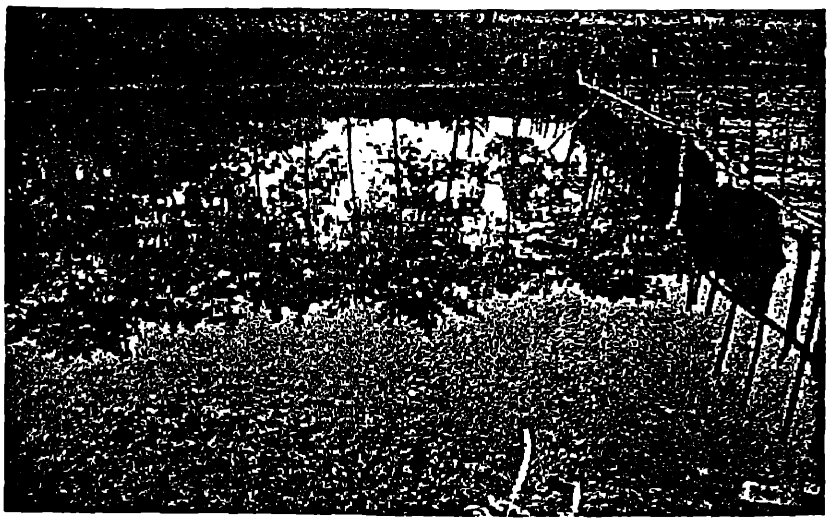


Figure 4.1 Bioremediation of Crude oil contaminated water





Before



After

Figure 4.2 Bioremediation of crude oil contaminated water with application of Oilzapper

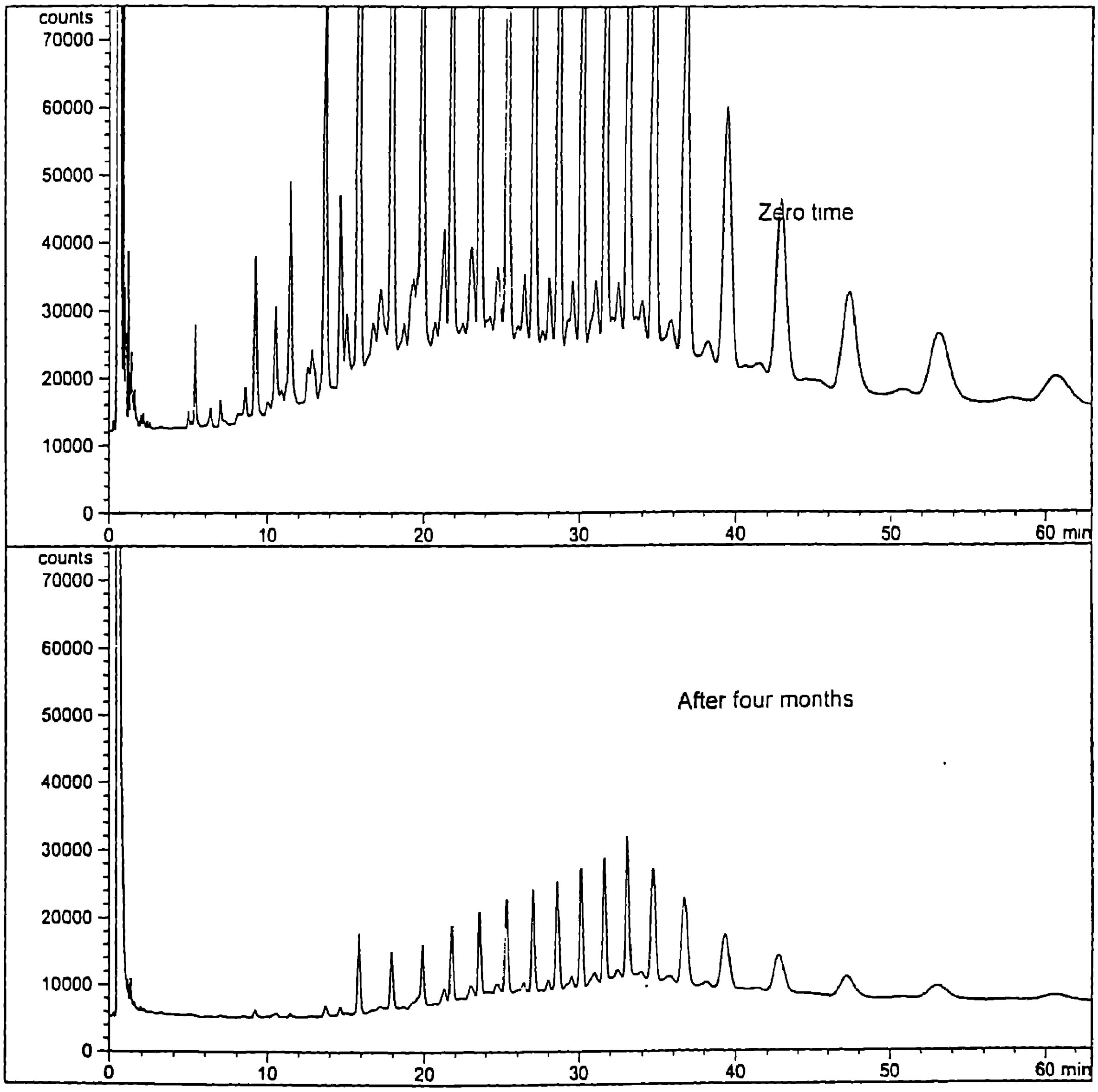


Figure 4.3 GLC fingerprinting of alkane fraction of crude oil collected from water pit near Joypur OCS

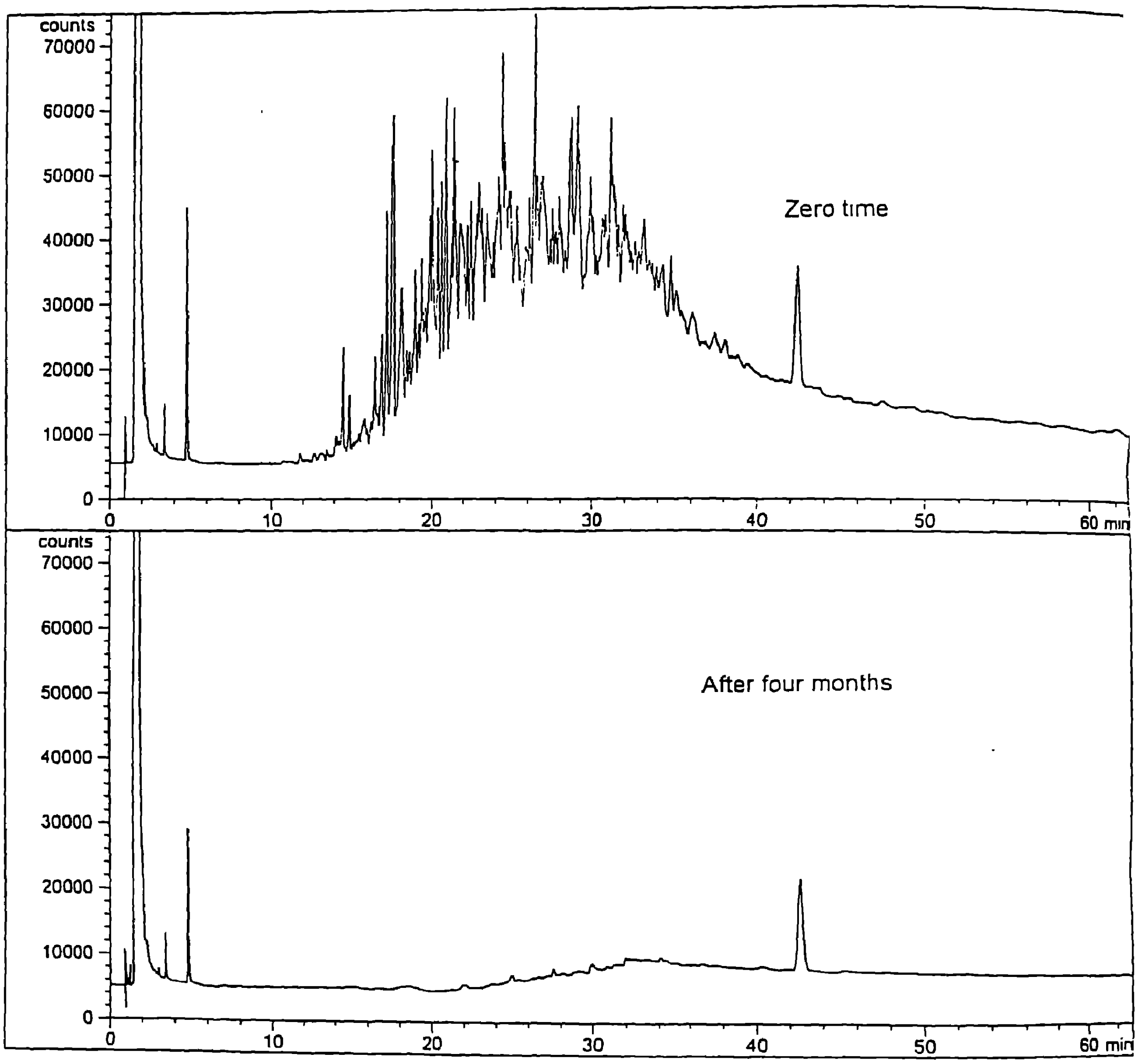


Figure 4.4 GLC fingerprinting of aromatic fraction of crude oil collected from water pit near Joypur OCS

5 Conclusion

- At Oil India Limited Duliajan, Bioremediation of crude oil contaminated land / water and treatment of oil sludge by Oilzapper was initiated in the month of November 1999. Initially bioremediation work was initiated in tank farm area and at Bowser unloading site near OCS 3. Later bioremediation of water pit contaminated with crude oil was also undertaken.
- At Bowser unloading site near OCS 3, soil was contaminated with crude oil spill. At this site, crude oil contaminated soil was treated with Oilzapper and nutrients. Treatment of this site with Oilzapper and nutrients resulted in the reduction of crude oil contamination in soil from 38.47% to 13.50% in four months.
- Initially Bioremediation of oily sludge in sludge pit 1, 5 and 6 were initiated in the month of November 1999. With encouraging results on bioremediation of oily sludge, two more sludge pits (pit 2 and 4) were undertaken for bioremediation of oily sludge.
- In sludge pit 1, total 96.9% oily sludge has been biodegraded by Oilzapper in one year.
- In sludge pit 5, with application of Oilzapper, 86.9% oily sludge has been biodegraded in one year.
- In sludge pit 6, 95% oily sludge has been biodegraded in one year by applying Oilzapper on oily sludge.
- In sludge pit 2 total 77.6% oily sludge has been biodegraded by Oilzapper in six months.
- In sludge pit 4 treatment of oily sludge with oilzapper revealed that 79.6% oily sludge has been biodegraded in six months.
- At Joypur OCS, water pit contaminated with crude oil was also undertaken for bioremediation. Oilzapper was applied in one-third portion of this pit, which resulted 99% biodegradation of TPH in fourteen months. Crude oil contaminated water in pit became clear and transparent as shown in figure 4.2.

6 Recommendations

- This project entitled "Bioremediation of Crude Oil and oily Sludge Contaminated soil and water" was a collaborative project between Tata Energy Research Institute, New Delhi and Oil India Limited, R&D Centre, Duliajan has been completed successfully. Results on bioremediation of tank sludge by Oilzapper are highly encouraging. With application of Oilzapper, crude tank sludge have been biodegraded at very fast rate. Now Oil India may adopt Oilzapper technology for treatment of crude tank sludge on regular basis.
- Oilzapper technology has also successfully demonstrated for clean up of crude oil contaminated water (including formation water) and land. Hence Oil India can use Oilzapper on regular basis for clean up of minor oil spills on land as well as on water. Oil India may procure Oilzapper from TERI, NewDelhi.